SOME FACTORS IN THE PURIFICATION OF SEWAGE BY THE ACTIVATED SLUDGE PROCESS

BY

GERALD CLIFFORD BAKER
A. B. University of Illinois
1917

THESIS

Submitted in Partial Fulfillment of the requirements for the

Degree of

MASTER OF SCIENCE

IN CHEMISTRY

IN

THE GRADUATE SCHOOL

OF THE

UNIVERSITY OF ILLINOIS

1919

UNIVERSITY OF ILLINOIS

THE GRADUATE SCHOOL

June 6, 1919.

I HEREBY RECOMMEND THAT THE THESIS PREPARED UNDER MY SUPERVISION BY Gerald Clifford Baker

ENTITLED Some Factors in the Purification of Sewage by the Activated Sludge Process

BE ACCEPTED AS FULFILLING THIS PART OF THE REQUIREMENTS FOR THE DEGREE OF Master of Science in Chemistry

W. F. Monfort
In Charge of Thesis

W. A. Noyes
Head of Department

Recommendation concurred in*

____
____
____

Committee on Final Examination*

*Required for doctor's degree but not for master's

TABLE OF CONTENTS

	page
I. Introduction	1
II. Historical	6
III. Experimental and Theoretical	
Colloidal Properties of Activated Sludge and Raw Sewage	10
Electrical Properties - Cataphoresis	10
Behavior of Colloids of Sewage and Activated Sludge when treated with various disinfectants	11
(a) Formaldehyde	11
(b) Chloroform	12
(c) Phenol	12
(d) Mercuric chloride	12
(e) Heat	12
(f) High Pressures	12
Effect of addition of clay colloids upon the colloidal state of sewage	13
Removal of sewage colloids by salting out agents	13
Effect of Disinfecting agents upon the purification of sewage by activated sludge	15
(a) Effect of formaldehyde	15
(b) " " chloroform	20
(c) " " phenol	21
(d) " " mercuric chloride	22
(e) " " heat	23

TABLE OF CONTENTS (cont.)

	page
(f) Effect of high pressures	24
Effect of aeration with different gases upon purification of sewage by activated sludge	26
Air, carbon dioxide, oxygen	26
Sulfur dioxide treatment	27
Effect of Mechanical agitation upon the purification of sewage by activated sludge	28
IV. Conclusions	30

SOME FACTORS IN THE PURIFICATION OF SEWAGE BY THE ACTIVATED SLUDGE PROCESS

I. INTRODUCTION

The activated sludge method of sewage disposal is still in the experimental stage. In brief the process consists in the aeration of sewage in the presence of accumulated sludge, which has itself been aerated and is enlivened with oxygen and bacteria. Aeration is followed by plain sedimentation with the removal of the resulting clear,supernatant liquid.

From the time the process was first discovered there has been much work carried on, both on the practical and theoretical phases of the problem. More attention has, however, been paid to the practical development of the process, but a few investigators have attempted to explain the nature of the purification, with the result that various theories have been suggested.

In their early work Ardern[1] and Lockett came to the following conclusions for the successful operation of the process, the purifying action of which they attributed to oxidation of the organic matter and nitrification of the ammonium compounds.

(a) In order that the final nitrification changes might take place without hindrance, it was necessary that the alkalinity or basicity of the sewage should be rather more than equal to the nitric acid resulting from the nitrification of the ammonium salts. We know, now however that this is not the case.

1. J. Soc. Chem. Ind. 33, (May 1914) , p. 151

(b) It was necessary that the activated sludge should be kept in intimate contact with the sewage during aeration.

(c) That the relation of the volume of the activated sludge to the volume of sewage treated was of importance, more especially in regard to the rate of nitrification.

They noted a rapid initial effect upon the oxidizable matter, both colloidal and criptalloid. The oxidation they divided into two types:

1. Oxidation of the carbonaceous material which was the initial effect.

2. Nitrification of the ammonium forms.

They noted, working with the fill and draw method, that if the activated sludge was called upon to treat further samples of crude sewage prior to the complete nitrification of the previous sample dealt with its activity was gradually decreased. They later showed that purification, however, is not directly dependent upon the nitrification.[2] They found the oxidation processes could be maintained within a fairly wide range of temperature. At temperatures below 10° C. a very marked deteroriation in the results was observed, especially with regard to the removal of colloidal matter. Nitrification was also practically inhibited. Temperatures as high as 30° C. did not seem to seriously interfere with the process.

They noted the high bacterial content of the activated sludge, and by reason of its nitrifying power suggested the

2. J. Soc. Chem. Ind. 36, Jan (1917)

presence of nitrifying organisms and the part that they might play in the purification processes. In the sludge they also isolated Protozoa, and suggested that they did not play an important part in the purification but rather indicated a particular condition of the activated sludge.

They found upon sterilization with steam that no purification whatever was effected but they recognized that the physical characteristics of the activated sludge might be seriously altered by the steaming process.

That the period of aeration was important was also noted. They state that "the period of aeration depends upon the strength of sewage treated and the degree of purification required." The advantages of diffused air over plain pipe aeration were recognized.

The next step in advance was also made by Ardern and Lockett when they came to the following conclusion[3]; that the success of the process was dependent upon the maintenance of the activity of the nitrifying organisms.

Melling[4] stated that while there was a material reduction in the oxidizable matters, the process appeared to be more physical than biochemical. He based his conclusions upon Salford sewage which had a sludge content of 9.67 per cent iron. He said, "The iron has possibly acted as a catalept in bringing down the colloidal matter."

3. J. Soc. Chem. Ind. 33, May (1914) p. 151.
4. J. Soc. Chem. Ind. 33, (1914) p. 1124.

Fowler and Mumford[5] suggested that "M-7", a peculiar organism precipitating iron as $Fe(OH)_3$ from iron salt solution might play some part in the purification.

At Salford it was also noted when copperas was added at the rate of 16 grains per gallon of sewage to accelerate the process that the purification was progressive.

Most investigators agree that bacteria play an important part in the purification process.

Russell[6] states that a combination of nitrifying bacteria with the other varieties present in sewage will purify sterile sewage but that nitrifying bacteria alone will not purify sterile sewage.

Hatton[7] suggests that the sludge must contain sufficient reducing and nitrifying organisms and adequate food and lodging to break down the organic matter into free ammonia and nitrogen and oxidize these to nitrates.

Contrary to early experiments Bartow[8] found that the worms aeolsoma hemprichi, often found in activated sludge, were not essential to the purification process.

Lederer[9] thinks the mechanical features of the process outweigh the biological features. He says "The higher putrescible colloids have simply been wiped out of suspension by continuous agitation." He also recognizes, however, that there is

5. J.of Royal San. Inst. 34, (1913-14) p. 497.
6. Master of Science Thesis "Biological Studies of Sewage Purification by aeration."
7. Eng. Rec. Oct. 16, 1915 p. 481.
8. J. Ind. and Eng. Chem. 7 (1915) p. 319
9. Eng. News, May 18, 1916.

some biological activity.

F. Dienert[10] attributes the purifying action of the activated sludge to the absorption of the organic matter by the precipitated $CaCO_3$, but this appears as unlikely.

This work was undertaken with the object of finding out if possible the various factors involved in the purification process and the relative importance of each in the whole process.

10. Compt. Rend. 165 (1917) 1116-7.

II. HISTORICAL

The idea of mechanical aeration in the purification of sewage was conceived of long before the activated sludge method of sewage disposal was discovered.

In 1882 aeration of sewage was tried out on London sewage by Dupre and Dibdin[11] and extensive experiments were carried out with the result that they found oxidation took place very slowly and little purification was effected.

The Massachusetts State Board of Health Experiment Station at Lawrence, Massachusetts; did some very comprehensive work in 1890 and subsequent years on the mechanical oxidation of sewage[12] but they came to the conclusion that such means of purification were not at all practical.

Other investigators confirmed the above conclusions - notably Mason and Hine in 1892[13] and Fowler in 1897.[14]

It was not until 1911 that any promising results were obtained by the mechanical aeration of sewage, when Black and Phelps[15] in New York blew air thru the sewage as it passed over a series of inclined wooden gratings. The sewage was in contact with the air for periods varying up to twenty-four hours. Altho oxidation was slight, the results were promising enough to cause

11. Royal Commission on Sewage Disposal. 1884 Vol. 2
12. Eng. Record, Feb. 7, 1914. p. 158.
13. Journal, Amer. Chem. Society, Vol. 14. p. 7.
14. Fifth Annual Report, 1897, Rivers Department, Manchester Corporation.
15. Report Concerning Location of Sewer Outlets and the Discharge of Sewage into New York Harbor (1911) 64-78.

Black and Phelps to recommend the construction of such a plant for Greater New York, but it was not adopted.

The next step in advance was discovered at the Massachusetts State Board of Health Experiment Station. Clark, Gage and Adams[16] found that on seeding sewage with algae growths that the oxidation of the organic matter was accelerated upon aeration. The algae were grown on slate plates, which were soon covered with spongy gelatinous growths which collected most of the suspended matter and much of the colloidal matter of the sewage.

In November 1912 Dr. Gilbert J. Fowler, of the University of Manchester, England visited the Massachusetts Experiment Station and was shown some successful bottle experiments in the purification of sewage thru which air had been bubbled. The bottles were emptied daily, leaving "growths" sticking inside, and were then refilled with fresh sewage. Stable and nitrified effluents resulted. Dr. Fowler on his return to England together with E. M. Munford[17] and later with Messrs. Edward Arden and W. T. Lockett[18] started similar experiments at Manchester, except that they retained the resultant sludge formed in the bottle, until it occupied about one-fourth its volume. The sludge in due time became a flocculent dark brown mass which settled out on standing, leaving a clear, highly nitrified effluent and one which was almost free from bacteria. Thus the activated sludge method of sewage disposal was discovered.

Since the discovery of the method development has been

16. Annual Report, Mass. St. Bd. of Health (1913) 45, 288-304.
17. Journal of Inst. Of San. Engrs., Mar. and Apr. issues 1916.
18. Jr. Soc. Chem. Ind. 33, 523-39; 1122-24 (1914).

gradual, because of the complexity of the factors involved. Experimental plants have been established in many American and European municipalities. The most notable of the English stations are at Worcester, Salford, Wakefield and Manchester. In the United States the most comprehensive work has been carried on at the experimental plants of (a) The Milwaukee Sewage-Testing Station, (b) Hygienic Laboratory of Washington, D. C., (c) Illinois State Water Survey, Urbana, (d) Cleveland Sewage-Testing Station, (e) Brooklyn Sewage-Testing Station, (f) Stockyards, Sanitary District of Chicago, (g) Brockton, Mass., (h) Houston, Texas, (i) Pasadena, Calif., (j) The Lawrence, Mass. Experiment Station and Baltimore, Md. Other noteworthy experimental plants are situated at Regina, Sask., Toronto, Canada and at Sydney, Australia.

In 1915 Milwaukee started the construction of the first working plant to be operated by this process. The plant was constructed to treat five million gallons of sewage daily. Due probably to the Great European war no statistics are available as to the operation of this plant on a large scale.

Experiments on a rather large scale were also carried out during 1916 at the Sewage Experiment Station of the Illinois State Water Survey under the direction of Dr. Edward Bartow. The operation results are published in the Illinois State Water Survey Bulletin, Series 14, 1917.

In 1915 the city of Houston, Texas adopted the activated sludge method for the disposal of the sewage of that city and for the past two years the plant has been treating the major portion of the sewage of that city with satisfactory results, but with

rather high costs.

It was early recognized that the disposal of the sludge was very important, and much work has been carried on to determine whether or not it has sufficient fertilizing value to warrant its use as a manure. Bartow and Hatfield[19] showed that activated sludge by virtue of its nitrogen and phosphate content, could be utilized as such a fertilizer, and carried on first, pot and later field experiments[20] proving it to be equal if not superior to other nitrogenous fertilizers of equal nitrogen content.

Nasmith and McKay[21] at the Toronto Experiment Station (1918) have substantiated Bartow's and Hatfield's results, and have pointed out that it would be an especially good fertilizer in truck gardening.

Since activated sludge is a good fertilizer there is placed a monetary value upon it, which gives promise to the future success of the process.

The most important practiced problem yet to be solved is the dewatering of the sludge. So far activated sludge has resisted the economical application of filter pressing, sand filtration and centrifuging.

The activated sludge method of sewage disposal seems suitable for those localities demanding a highly oxidized effluent, and where the air can be compressed at a moderate cost.

19. Jour. Ind. and Eng. Chem. Vol 8 No. 1 p. 17 Jan. (1916).
20. Amer. Soc. for Mun. Imp. (1918) 225-257.
21. Jour. Ind. and Eng. Chem. Vol. 10 No. 5 p. 399 May 1918.

III. EXPERIMENTAL AND THEORETICAL.

Colloidal Properties of Activated Sludge and Raw Sewage.

Preliminary experiments were run in an attempt to determine the colloidal properties of activated sludge and raw domestic sewage.

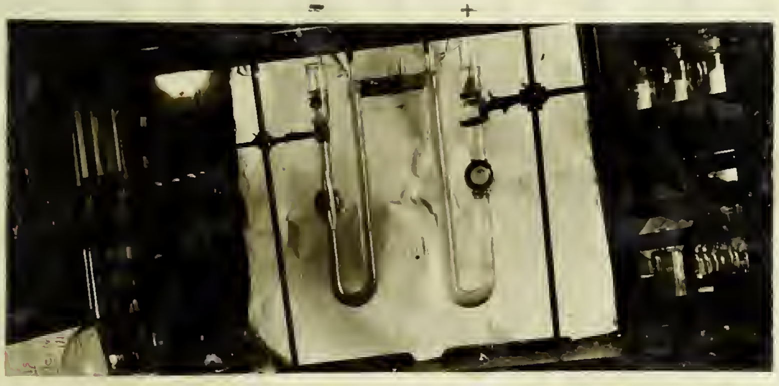
Electrical properties - Cataphoresis.

Under the influence of a fall of potential almost all colloids migrate either to the cathode or the anode. When the particles move thru the solution the phenomenon is called cataphoresis. The suspended particles take on a charge either positive or negative. These charges determine the direction in which the particles travel in the current.

In the first series of experiments a cataphoresis was run on activated sludge in an attempt to determine the charge of the colloids. The apparatus used consisted of two transference number tubes connected as shown in accompanying plate. The electrodes were of platinum gauze and the current used was 110 volts direct current. The apparatus was filled with activated sludge based upon a thirty minute settlement period. In the runs with activated sludge it was found there was a general migration towards the cathode, but the results were rather unsatisfactory, since after about six to eight hours the sludge became septic and rose to the top of the liquid in each tube, and it was necessary to abandon the cataphoresis after that period, but from the work it is

evident that the greater mass of the activated sludge colloids are positively charged.

The above experiment was repeated using raw domestic sewage collected from the main Champaign sewer at the Power Plant of the University of Illinois. Excellent results were obtained in these runs, some of which lasted as long as ninety hours. Here again the general migration was towards the cathode, showing that at least the greater number of the colloids were positively charged. The accompanying plate clearly shows the results.



This run lasted ninety hours.

<u>Behavior of colloids of sewage and activated sludge when treated with various disinfectants</u>

In the first work an attempt was made to sterilize both the sewage and activated sludge, without changing the colloidal properties of either in the hope that it might be proven what effect bacteria played in the purification process, and how much was due to physical effects.

(a) The first disinfecting agent used was formaldehyde in varying amounts. It was found that sterility in both the sludge and sewage was produced by the addition of enough formaldehyde to make a .15% solution if let stand for a period of forty-eight hours. In case of both the sewage and sludge macroscopically and electri-

cally the colloidal state was apparently undisturbed as shown by cataphoresis experiments.

(b) Attempted disinfection with chloroform was a failure in case of both the sewage and sludge, but amounts as high as ten cubic centimeters per liter for forty-eight hours did not apparently change the colloidal state of either the sewage or sludge, altho the sludge at the end of that time had started to become septic.

(c) Likewise it was found that Phenol did not materially change the colloidal properties of either the sewage or sludge, when added in amounts to give a saturated (5%) solution, altho there was only slight movement towards the cathode sterility was not produced.

(d) Sterility was produced by mercuric chloride one part in one thousand in both sewage and sludge in forty-eight hours, but in each case the colloidal properties were changed. The mercuric chloride was put in solution by aeration and almost immediately a coagulation of the colloids took place in both the sewage and sludge. A cataphoresis was not run as it was known that there had been a change in the colloidal state.

(e) Heat was next applied. Both sewage and activated sludge were heated at 60° for one hour. In the case of the sewage some coagulation took place, and the colloidal state was changed. The activated sludge rose to the top of the solution. Cataphoresis not run as it was evident that there had been a change in the colloidal properties. Sterility was not obtained.

(f) High pressure was applied in the form of liquid car-

bon dioxide. The sewage and sludge were placed in a small pressure tank which had been previously lined with parafine. This tank was connected to a tank of liquid carbon dioxide and the full pressure (56-57 atmospheres at 20°) was turned on. The sewage and sludge was thus subjected to this pressure for twenty-four hours. At the end of which time the pressure was released as gradually as possible. Apparently there had been very little change in the colloidal properties of either the sewage or the activated sludge. Sterility was not produced.

Effect of addition of clay colloids upon the colloidal state of sewage.

Very finely divided clay was added to sewage in amounts equivalent to the dry matter in that amount of sludge required in its purification by the activated sludge process, based upon a 25% sludge, containing 95% moisture. Analyses by Hatfield[22] has shown that the water content of activated sludge is 95%. The sewage so treated was placed in a 2500 cc bottle and gently shaken up and the contents allowed to settle for twenty-four hours. A major portion of the sewage colloids were removed from the solution.

Removal of sewage colloids by salting out agents.

Two common salting out agents were used; ammonium sulfate and sodium chloride. Upon treating sewage by saturation with ammonium sulfate it was possible to salt out most of the colloids within two hours. By saturation with sodium chloride there was some salting out action, but only a fractional part of the colloids were

22. Jour. of Ind. and Eng. Chem. Vol. 7 No. 4 p. 318. Apr. 1915.

removed. This would tend to show a selective action, which might indicate the albuminous character of the sewage colloids.

Almost from the beginning of sewage purification the importance of microorganisms has been recognized. Upon filtration of sewage Dunbar and Calvert [23] attribute the clarification to an absorption of the colloids by gelatinous films formed around the sand grains and which contain many bacteria and other low forms of life. These gelatinous coatings become thicker and thicker and it is assumed that they have a honey-comb structure, and therefore possess an exceedingly large surface. They have an internal as well as an external surface and can sbsorb large quantities of gases, as oxygen, as well as many organic and inorganic substances. After absorption oxidation continues and the organic matter is partially broken down.

It is possible that such a process is taking place in the purification of sewage by activated sludge since the sludge is flocculent and porous, in character, and absorbs the sewage colloids, possibly by an accumulation of the gelatinous forms, caused by surface attraction and direct suction. The direct suction is probably produced when the absorbed oxygen of the sludge is used up and replaced by fresh oxygen from the air in the pores. This causes a partial vacuum in the pores of the sludge and the surrounding air is drawn in with considerable energy.

23. Dunbar and Calvert - Principles of Sewage Treatment.140-149.

Effect of disinfecting agents upon the purification of sewage by activated sludge.

During the progress of this work the original experimental activated sludge plant of the Illinois State Water Survey has been kept in operation. The plant which is located in the basement of the University power plant, has been described by Bartow and Mohlman[24] in one of their early publications. The sludge and sewage used in the laboratory experiments has been obtained from this plant. The laboratory experiments were carried out in glass cylinders, 24 inches in height, 6 inches in diameter , with a capacity of about 3 gallons. In all the aeration experiments 7500 cc of sewage and 2500 cc of activated sludge, based upon a thirty minute settlement period, have been used. Compressed air from the University supply was blown into the mixture thru a Hodkinson type filter strainer. Check results with untreated sludge and sewage have been run in all cases. In all the experiments the decrease in the turbidity has been taken as a measure of the degree of purification, based upon a thirty minute settlement period after aeration. The degree of sterility has been determined by plating out on plain agar with a digestion period of 48 hours.

(a) Effect of Formaldehyde

It was determined by preliminary experiments that the addition of enough formaldehyde to both sewage and sludge to make a .15% solution, would produce sterility in either case in 48 hours.

24. Jour. of Ind. and Eng. Chem. Vol. 8 No. 1 p. 15 Jan. 1916.

Seventy-five hundred cc. of sewage and 2500 cc. of sludge were so sterilized and placed in the aerating cylinder described above, and aeration carried out with the following result.

Table I.

	Sterilized	Check
Turbidity of original sewage	250 ppm.	250 ppm.
" at end first hours aeration	270 "	75 "
" " " second " "	330 "	65 "
" " " third " "	380 "	50 "
" " " fourth " "	450 "	40 "

Note - increased aeration gave an increased turbidity with the sterilized mixture.

After forty hours aeration by plating out some of the solution it was found that the sterility had been maintained.

Here apparently the power of purification had been destroyed, and the next attempt was to seed this same sterilized mixture with types of bacteria known to be present in sewage and activated sludge and see if purification could be obtained. The first attempt was to seed with the B. subtilis group of organisms. After digestion for 24 hrs. the count on agar was 5000 per cc. Aeration was then carried out with the following result.

Table II.

Turbidity at start of aeration	210 ppm. (settlement for 30 hrs.)
" " end 2 hours "	500 "
" " " 4 hours "	850 "
" " " 8 " "	950 "

To a similarily treated solution, nitrifiers and the other typical sewage flora were added in the form of a small amount of activated sludge (100 cc.) and digested for 1 hour, when aeration was carried out with the following results.

Table III.

Turbidity at start of aeration	450 (settlement for 15 hours).
" " end 2 hours "	450 ppm.
" " " 5 " "	400 "
" " " 10 " "	280 "
" " " 20 " "	200 "
" " " 30 " "	120 "
" " " 35 " "	40 "
" " " 40 " "	less than 25 "

The next attempt was to purify sewage by dried activated sludge. The sludge used was some that had been dried by Hatfield in 1917. The sludge was ground up very finely and placed in 2500 cc of distilled water. The amount used was that amount equivalent to the dry matter in an equal quantity of fresh activated sludge (125 g in 2500 cc). This prepared solution was added to 7500 cc of raw sewage in the aeration cylinders and air blown thru the mixture. The following table shows the results.

Table IV.

			Check
Turbidity of sewage at start of aeration		170 ppm.	170 ppm.
" at end 4 hours	"	280 "	40 "
" " " 10 "	"	320 "	
" " " 20 "	"	320 "	
" " " 30 "	"	330 "	
" " " 40 "	"	320 "	

The sludge did not regain any of its flocculent properties and no purification could be effected. The next test was then to see if the sewage itself contained the proper bacterial flora necessary for purification. Fresh activated sludge was sterilized with a resulting solution of .15% formaldehyde. This sludge (2500 cc) after standing 48 hours was then added to 7500 cc in the aeration cylinder and air blown thru. The results are recorded below.

Table V.

Turbidity of sewage at start of aeration		250 ppm.
" at end of 1st hrs.	"	260 "
" " " " 5th "	"	340 "
" " " " 10th"	"	380 "
" " " " 24th"	"	480 "
" " " " 30th"	"	660 "
" " " " 36th"	"	540 "
" " " " 50th"	"	320 "
" " " " 80th"	"	180 "
" " " " 95th"	"	110 "
" " " " 100th"	"	85 "
" " " " 120th"	"	40 "

Thus it was shown that the sewage itself contains the necessary bacterial flora for the purification process, and that the sludge must itself be in the proper physical condition, and that the colloidal state of the sewage and sludge had not been materially changed by the treatment with formaldehyde and by over-aeration.

(b) Effect of chloroform.

After preliminary experiments it was shown the chloroform would not sterilize either the sewage or the sludge when added in amounts sufficient to produce saturation. Fifteen cc of chloroform were added to 2500 cc. of the sludge and 45 cc. to 7500 cc. of sewage and let stand for 48 hours, at the end of which time they were placed in the aeration cylinder and air blown thru with the following results.

Table VI.

		Check
Turbidity of sewage at start of aeration	250 ppm.	255 ppm.
" at end 1st hours "	250 "	90 "
" " " 2nd " "	280 "	60 "
" " " 3rd " "	250 "	50 "
" " " 4th " "	260 "	30 "
" " " 10th " "	260 "	
" " " 20th " "	290 "	

The chloroform had evidently killed most of the bacteria except spore forms. The bacteria count on agar before aeration was

(a) sewage 150 per cc.

(b) activated sludge 400 " "

Fresh activated sludge (100 cc) was added to the above mixture and aerated with the following results.

Table VII.

Turbidity at start of aeration	240	ppm.
" " end 4 hours "	220	"
" " " 10 " "	140	"
" " " 15 " "	60	"
" " " 18 " "	40	"

This again shows that the colloidal state was not destroyed by the treatment with chloroform, and also shows a possible function of the spore-forming bacteria in the purification process, since continued aeration did not bfeak up the activated sludge and give increased turbidity.

(c) <u>Effect of Phenol</u>

By the addition of sufficient phenol to both activated sludge and sewage to produce a 2.5% solution it was found that sterility could not be obtained - bacterial counts after treatment showed

sewage 120 per cc.

Activated sludge 395 " "

Results of aeration (2500 cc treated sludge - 7500 cc treated sewage.)

Table VIII.

		Check
Turbidity of sewage at start of aeration	500 ppm.	500 ppm.
" at end 1st hours "	1000 "	150 "
" " " 4th " "	1200 "	60 "
" " " 8th " "	1400 "	
" " " 20th " "	1600 "	
" " " 30th " "	1650 "	

Addition of (100 cc) activated sludge gave no purification upon additional aeration, nor did the addition of 500 cc additional activated sludge produce clarification, hence the theory suggests itself that the colloidal state had been changed which could not be detected by the cataphoresis experiments. It is probable that there might have been a slight attack upon the albuminous matter, and this also evidences that the physical state of the activated sludge plays a very important part in the clarification, altho by inspection the flocculent state of the activated sludge did not seem to have been altered.

(d) Effect of Mercuric Chloride

Mercuric chloride was added to sewage to make a solution of 1 part in a 1000. The mixture was aerated to facilitate the solution of the $HgCl_2$. Coagulation almost immediately took place. After aerating for one half hour and letting settle for thirty minutes a turbidity of 10 was obtained. The turbidity of the original sewage was 300 ppm. Sterility was produced in 48 hrs.

The mercuric chloride apparently coagulated the albuminous matter of the sewage and produced clarification, hence the question is brought up, is clarification in any way connected with the coagulation of the albuminous matter?

(e) Effect of Heat.

Seventy-five hundred cc of sewage and 2500 cc of activated sludge were heated in a water bath at 60° for one hour. Slight coagulation of the sewage occurred, while the activated sludge rose to the top of the mixture. Sterility was not produced-counts

(a) sewage 2200 per cc

(b) activated sludge 5000 " "

Results of aeration experiments.

Table IX.

		Check
Turbidity of sewage at start of aeration	340 ppm.	340 ppm.
" at end 1st hours "	420 "	180 "
" " " 2nd " "	510 "	95 "
" " " 3rd " "	590 "	75 "
" " " 4th " "	625 "	45 "
" " " 10th " "	650 "	

Fresh activated sludge (100 cc) then added and aeration continued after letting stand for one hour

Results

Table X.

Turbidity at start	590 ppm.	
" " end 1st hours aeration	590 "	
" " " 4th " "	600 "	
" " " 10th " "	580 "	
" " " 25th " "	390 "	
" " " 33th " "	280 "	
" " " 45th " "	280 "	

Apparently the colloidal state of the activated sludge had been so effected that complete clarification could not be obtained, altho after the introduction of the fresh activated sludge some purification was effected.

(f) Effect of High Pressures (Carbon Dioxide).

In thése experiments 1000 cc activated sludge and 3000 cc sewage were placed in a small pressure tank which was then connected to a liquid carbon dioxide cylinder, and the full pressure turned on. The mixture was subjected to this pressure for four hours at the end of which time the pressure was released as gradually as possible. The mixture which had assumed a greenish color due to the iron which had gone into solution by the action of CO_2 upon the cylinder was then submitted to aeration with following results- Sterilization was not produced - agar count - 530 per cc.

Table XI.

		Check
Turbidity of sewage at start	200 ppm.	200 ppm.
" at end of 1st hours aeration	80 "	135 "
" " " " 3rd " "	90 "	80 "
" " " " 4th " "	90 "	20 "

It was thought that probably the iron that the mixture had taken up due to action of the CO_2 upon the walls of the cylinder might have had something to do with the action on aeration. Accordingly the above experiment was repeated after the tank had been lined with paraffin. The results of this aeration are given below.

Table XII.

		Check
Turbidity of sewage at start	200 ppm.	200 ppm.
" at end of 2nd hours aeration	220 "	100 "
" " " " 3rd " "	200 "	70 "
" " " " 4th " "	210 "	30 "
" " " " 10th " "	400 "	
" " " " 25th " "	650 "	
" " " " 40th " "	800 "	

Five hundred cc fresh activated sludge was then added and the aeration continued with the following results.

Table XIII.

Turbidity at start of aeration	740 ppm.
" " end of 4 hours "	720 "
" " " " 8 " "	510 "
" " " " 23 " "	140 "
" " " " 25 " "	100 "
" " " " 30 " "	50 "

Again clarification was produced after seeding with the proper bacterial flora. In aeration after the treatment there is noted an increasing turbidity even with the spore-forming bac-

teria present, which was not the case with the sewage treated with chloroform. It is possible that the CO_2 had temporarily changed the colloidal state due to a change of the hydrogen ion concentration of the solution. The CO_2 evidently was driven on aeration and after seeding with activated sludge, clarification was made possible.

Effect of aeration with different gases upon purification of sewage by activated sludge.

In the experiments which followed an attempt was made to prove whether it was only the intimate contact of the activated sludge with the sewage that produced the purification, or whether the gas used in the aeration played any part. Accordingly aerations were run with CO_2, with the result that very little purification resulted. Then it was thought if the oxygen of the air was responsible for the purification, that oxygen itself might cause more rapid purification accordingly such aerations were run by the method previously described. The results are shown in the following table.

Table 14.

	Oxygen	Air	Carbon Dioxide
Turbidity of sewage at start of aeration	210 ppm.	210ppm.	210 ppm.
" at end of 1 hours "	80 "	90 "	130 "
" " " " 2 " "	45 "	65 "	160 "
" " " " 3 " "	45 "	55 "	170 "
" " " " 4 " "	40 "	55 "	190 "
" " " " 18 " "	--	--	510 "

Thus it would seem that the oxygen was directly responsible for the purification. The slight reduction of the turbidity at the end of the first hours aeration with CO_2 may have been due to the oxygen dissolved in the sewage.

Sulfur Dioxide Treatment.

It was thought the activated sludge process might be comparable to the Miles acid treatment. Accordingly an attempt was made to determine whether there was a change of hydrogen ion concentration during the purification process. Fresh sewage was aerated for 5 days and the hydrogen ion concentration run on each successive day. The results were as follows:

Sewage As collected	Ph = 7.6
After 24 hours aeration	Ph = 8.0
" 48 " "	Ph = 8.2
" 72 " "	Ph = 8.1
" 96 " "	Ph = 8.2
" 120 " "	Ph = 8.2

Same sewage when treated with activated sludge gave following result.

Effluent Ph = 7.7

Filtered activated sludge= 7.4

The method used in determing the hydrogen ion concentration was a modification of the method of Levy, Rowntree and Marriott[25]. Three cc. of sewage to be tested were placed in a glass tube 100 by 10 mm. inside measurements and .2 cc of a .01 per cent phenolsul-

25. Archives of Internal Medicine. 16, pp. 389-405.

phonethalein indicator added and thoroughly mixed. The color produced was compared with a series of standards prepared from this indicator.

It was also shown that the activated sludge method was not comparable to the Miles acid process by blowing SO_2, thru a mixture of activated sludge and sewage, and then aerating. Purification could not be accomplished and the sludge was partly disintegrated.

Evidently acid production by bacteria is not a factor in the removal of the colloidal matter from sewage in the activated sludge process.

Effect of mechanical agitation upon the purification of sewage by activated sludge.

These experiments were also run to see if oxygen was necessary for the purification, and to see whether intimate contact of the sewage and activated sludge would produce clarification in its absence. Two sets of experiments were run, one with gentle shaking every 15 minutes for three and one half hours then letting the mixture settle for thirty minutes. The other was run with vigorour shaking for three and one half hours with thirty minutes settlement. These experiments were carried out in 2500 cc bottles, containing 400 cc activated sludge and 1600 cc sewage.

Table XV.

	Vigorous shaking	Gentle shaking	Check Alteration
Turbidity of sewage at start	320	320	320
" At end 4 hours	500	150	50

By the gentle shaking some clarification was produced,

probably due to some oxidation by the dissolved oxygen of the sewage, and also due to the mechanical absorption of the sewage colloids by the activated sludge. The vigorous shaking seemed to have partially broken up the floccules of activated sludge.

During the progress of this work, some difficulties have arisen in the successful operation of the experimental activated sludge plant from which all the activated sludge and sewage was obtained for the laboratory experiments. At times the sewage was contaminated with large quantities of oil which was pumped into the tank with the sewage. During these periods purification was greatly interfered with, but it was found that by skimming off the oil, much better clarification could be obtained. It is possible that the oil may have absorbed the oxygen and may have in some way affected the colloidal state of the sewage.

CONCLUSIONS

1. The purification of sewage by activated sludge is due to oxidation.
2. The oxidation is largely carried on by aerobic bacteria.
3. Sewage itself contains the bacterial flora necessary for purification independent of the types present in activated sludge.
4. The biological oxidation is attended by absorption of the sewage colloids by the activated sludge.
5. The activated sludge process is not comparable to the Miles acid treatment since purification is not dependent upon acidity.
6. For good clarification the activated sludge must be kept in intimate contact with the sewage, and the sludge itself must be in proper physical condition.
7. The removal of the colloids from sewage by activated sludge is directly dependent upon bacteria, and may be due to a secretion of enzymes by the bacteria.
8. The colloids of sewage are for, at least, the greater part positively charged and may be largely removed by the introduction of negatively charged colloids and by salting out with ammonium sulfate.

ACKNOWLEDGMENT

The writer desires to express his gratitude to Professor W. F. Monfort, under whose direction this investigation has been carried out, for the unfailing enthusiasm and helpful suggestions he has offered during its progress. He also wishes to thank Lieut. W. D. Hatfield for his suggestions during the early part of the work, and G. C. Habermeyer for his cooperation at all times.